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ABSTRACTS FOR ORAL PRESENTATION

ABSTRACTS FOR POSTER PRESENTATION

Abstracts for Oral Presentation

Keynote Presentation

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Hypothalamic pituitary adrenal (HPA) axis: Regulation of immunity and role in susceptibility to autoimmune, inflammatory and infectious diseases

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Neural and neuroendocrine responses including the HPA axis, sympathetic and parasympathetic responses regulate immune responses and play an important role in susceptibility and resistance to inflammatory and infectious diseases. HPA axis activation inhibits inflammation through the anti-inflammatory and immunomodulatory actions of glucocorticoids that shift cytokine production from a TH1 to a TH2 pattern and enhance delayed type hypersensitivity. Interruptions of the HPA axis whether genetic, surgical or pharmacological, render inflammatory resistant hosts susceptible to inflammatory disease. Over-activation of this axis, as in chronic stress, enhances severity of infections, through the immunosuppressive effects of the glucocorticoids. The sympathetic, parasympathetic and peripheral nervous systems modulate inflammation at regional or local levels, releasing generally pro-inflammatory neurotransmitters. The association of a blunted HPA axis with autoimmune/inflammatory disease occurs across species, strains and diseases, and in human inflammatory illness, including rheumatoid arthritis, SLE, Sjogren's syndrome, allergic asthma, atopic skin disease and irritable and inflammatory bowel disease. Tissue resistance to glucocorticoids resulting from polymorphisms, mutations or dysfunction of the glucocorticoid receptor (GR) is also associated with autoimmune/inflammatory disease. Bacterial toxins can repress the GR and other nuclear hormone receptors, suggesting potential new mechanisms for shock and inflammatory sequelae of bacterial infections, and new avenues for therapy of these conditions. Understanding these connections can help explain how stress exacerbates inflammatory and immune mediated skin diseases.

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Neuroanatomy of the skin

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The skin is equipped with an effective communication and control system designed to protect the organism in a constantly changing environment. For this purpose a dense network of highly specialized afferent sensory and efferent autonomic nerve branches occurs in all cutaneous layers. The sensory system contains receptors for touch, temperature, pain, itch and various other physical and chemical stimuli. The information is either processed in the central nervous system or may directly elicit an inflammatory reaction by antidromic propagation of the impulses. The autonomous system plays a crucial role in maintaining cutaneous homeostasis by regulating vasomotor functions, pilomotor activities, and glandular secretion. The effector function of a nerve may be determined by the secreted neuropeptides and the corresponding receptors of the target structures. The close contact of neural structures with various immune cells is the anatomic basis for a strong interaction between the nervous- and the skin immune system.

Stress, Neural Modulation and Cutaneous Immunity I

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Overview of cutaneous immunity

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The skin is poised to react to microbial invaders with a variety of mechanisms. Innate immune responses are rapid and inflammatory but relatively nonspecific. However, they initiate and localize the adaptive immune response, which engages highly specific B cells (antibodies) and T cells (inflammation and cytotoxicity). The presentation of antigens to naïve T cells by specialized dendritic cells (Langerhans cells) that mature upon emigration to the draining lymph node while carrying antigen, is a particularly potent function of the skin. This initiates a skin-lymph node-blood recirculation-skin sequence, whereby expanded and activated effector T cells can become re-activated in the skin by less stringent antigen presenting cells, including immature DC and macrophages, and coordinate inflammatory reactions. The range of inflammatory responses of the skin includes reactions involving Th1 cells, Th2 cells, and Th-IL-17 cells, in balance with control mechanisms such as Tregulatory cells. Infectious disease responses, tumor immune responses, and autoimmune/inflammatory conditions such as contact & atopic dermatitis, psoriasis, T cell lymphoma, lupus or pemphigus invoke these mechanisms in various permutations that exemplify the extraordinary scope of the skin immune system, which also must maintain a quiescent state while harboring commensal organisms. Multiple inputs into skin control of inflammation derive from the neurocutaneous axis, and create a pathway for stress responses to influence cutaneous immunity.

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Nerve-derived mediators and cutaneous immunity

R Granstein *Department of Dermatology, Weill Medical College of Cornell University, (New York, New York, USA)*

Psychological state and neurologic influences are believed to regulate cutaneous immunity. For example, there is evidence that inflammatory skin diseases such as atopic dermatitis and psoriasis worsen with stress. Additionally, recent data from both human and animals studies suggests that psychological stress can influence cutaneous immunity. In support of the concept that the nervous system can regulate immunity in the skin, it has been found that epidermal Langerhans cells (LC), believed to play a role in the initiation and regulation of cutaneous immunity, are anatomically-associated with epidermal nerves. LCs have been found to express receptors for several neuropeptides including calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating peptide (PACAP), vasoactive intestinal peptide (VIP) and gastrin-releasing peptide. Additionally, LC express several adrenergic receptors and have been found to express receptors for purinergic agonists. Studies have been performed to evaluate the effects of nerve-derived products on LC function. CGRP, PACAP and VIP all inhibit antigen presentation and modulate cytokine expression. Similarly, exposure of LC *in vitro* to epinephrine or norepinephrine inhibits antigen presentation. Furthermore, injection of naive mice *in vivo* with CGRP, PACAP or epinephrine inhibits the ability of mice to be fully immunized to a hapten painted at the site of injection for CGRP and PACAP or even at a distant site for epinephrine. The long-lived ATP analog, ATPγS, enhances antigen presentation by LC in the presence of the TLR agonist lipopolysaccharide. As a whole, these data demonstrate the capability of nerve-derived factors to regulate cutaneous antigen presentation and suggests a locus of interaction between the nervous system and the immune system within the skin.

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Neuropeptides and dendritic cells

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Neuropeptides abundant in skin and lymphoid organs act as immunoregulators, affecting both innate and adaptive immune responses. Tolerogenic dendritic cells (DCs) play an important role in maintaining peripheral tolerance through the induction/activation of regulatory T cells (Treg). Endogenous factors contribute to the functional development of tolerogenic DCs. We reported recently that two known immunosuppressive neuropeptides, the vasoactive intestinal peptide (VIP) and the pituitary adenylate cyclase-activating polypeptide (PACAP), contribute to the development of bone marrow-derived tolerogenic DCs *in vitro* and *in vivo*. The VIP/PACAP-generated DCs are $CD11c^{low}CD45RB^{high}$, do not upregulate CD80, CD86, and CD40 following LPS stimulation, and secrete high amounts of IL-10. The induction of tolerogenic DCs is mediated through the VPAC1 receptor and protein kinase A, and correlates with the inhibition of IkB phosphorylation and of NFkBp65 nuclear translocation. The VIP/PACAP-generated DCs induce functional Treg *in vitro* and *in vivo*. The VIP/DC-induced Treg resemble the previously described Tr1 in terms of phenotype and cytokine profile, suppress primarily Th1 responses including delayed-type hypersensitivity, and transfer suppression to naïve hosts. The effect of VIP/PACAP on the DC-Treg axis represents an additional mechanism for their general anti-inflammatory role, particularly in anatomical sites which exhibit immune deviation or privilege.

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Alpha melanocyte stimulating hormone and the skin

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Melanocortins are structurally related bioactive peptides which are produced by many extra-neural tissues including the skin. All of the melanocortins (α, *β*, and γ-melanocyte-stimulating hormone and adrenocorticotropin) have melanotropic activity but can elicit many other effects on skin cells. On the basis of *in vitro* and *in vivo* findings αMSH and αMSH derived peptides such as KPV and KPT have been shown to regulate immune and inflammatory responses, hair follicle growth, exocrine gland activity and extracellular matrix composition. Accordingly, these peptides successfully have been used in murine models of allergic contact dermatitis, allergic airway inflammation, and dextrane sulphate induced colitis. The effects of αMSH mainly are mediated by melanocortin receptors among which the melanocortin-1 receptor (MC1-R) is most ubiquitously expressed by human skin- and immunocompetent cells. Simultaneous expression of melanocortins and their receptors suggest a complex autocrine and/or paracrine regulatory network whose disruption invariably affects skin homeostasis. Expression of melanocortin receptors on various skin cell types further indicates novel pharmacological targets for the treatment of skin disorders as well as inflammatory, allergic and autoimmune diseases.

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Neuroreceptors and mediators

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Stress may activate the neurocutaneous axis and influence skin structures especially inflammatory cells, mast cells and nerve fibers. Many stress-associated mediators such as epinephrine and corticotropin-releasing hormone bind to skin nerve fibers or mast cells. As a result, mast cell degranulation, activation of neuroreceptors on sensory nerve fibers and neurogenic inflammation occur. Neuromediators released from sensory nerve fibers such as substance P further aggravate the stress-induced responses by binding to mast cells and inducing mediator release. Current investigations disclosed that many of the involved neuroreceptors are co-expressed, interact and potentate their effects. As a consequence, stress-mediated peripheral sensitization of nerve fibers occurs which leads to prolonged pain and itch as well as allodynia and alloknesis. For example, the receptor TrkA and its ligand nerve growth factor (NGF) sensitize the vanilloid (capsaicin) receptor TRPV1. Inflammatory mediators such as bradykinin and prostaglandin maintain these effects by permanent stimulation of nerve fibers. The opposite, neuroreceptors such as mu-opioid receptor, or cannabinoid receptors may interrupt the stress-induced responses and therefore represent targets for new therapeutical modalities. These observations have huge clinical implications. Peripheral sensitization of nerve fibers in atopic dermatitis is part of the severe itch associated with this disease and – according to clinical observation – may be worsened by stress. In sum, stress may influence skin functions such as pain, and itch, and contributes to the worsening of inflammatory diseases.

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Effect of acute stress on skin mast cell-dependent vascular permeability

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Many skin disorders, such as atopic dermatitis, psoriasis and chronic urticaria are associated with increased numbers of activated mast cells and are worsened by stress; however, the mechanism underlying these processes is not understood. Corticotropin-releasing hormone (CRH) is secreted under stress in the skin, where it induces mast cell activation and vascular permeability. Intradermal administration of CRH induces Evans blue extravasation that is almost comparable to that of neurotensin (NT). Pretreatment of injection sites with the NT receptor antagonist SR48692 prior to CRH blocks this effect. CRH-induced vascular permeability is also diminished in NT −/− mice, implying that NT is necessary for the effect of CRH. CRH and NT precursor mRNA are expressed in dorsal root ganglia (DRG) and skin by RT-PCR. The effect of both CRH and NT is absent in W/W^v mast cell deficient mice; however, only a fraction of skin mast cells express CRH receptors as shown by FACS analysis of CRH and c-kit double-positive disaggregated mouse skin mast cells. Human cultured mast cells express both CRHR-1 and CRHR-2, activation of which leads to selective release of vascular endothelial growth factor (VEGF) and IL-6, respectively. Skin biopsies from patients with chronic urticaria have increased expression of CRHR-1, while those from psoriasis have decreased expression of CRHR-1, implying over stimulation, a finding supported by high serum CRH in the latter patients. These findings suggest that CRH, together with NT, induces skin vascular permeability through mast cell activation and could participate in the pathogenesis of skin disorders exacerbated by stress. These new targets could be useful for the development of novel therapeutic approaches involving CRHR antagonists (US Patent No. 6,020,305 awarded to TCT) and mast cell activation blockers (Supported by NIH grant R01 AR47652 to TCT).

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Stress-induced alterations in mouse T lymphocytes: Genomic analyses

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Stress is believed to influence clinical outcomes that are mediated by changes in immune reactivity; in particular, increased susceptibility to upper respiratory tract infections. While stress can manifest in reduced immune cell number or function, recent reports suggest that stress may also enhance an inflammatory response. These bidirectional effects of stress on the immune system are interesting and the mechanisms through which stress alters immune reactivity on a cellular level remains to be fully elucidated. It has been hypothesized that stress may alter immune function by inducing damage to nuclear material within an immune cell and/or alter the capacity of immune cells for DNA repair. Either cellular event would have consequences for immunity as it would likely result in apoptosis of immune cells. We investigated gene expression in T lymphocytes in BALB/c mice subjected to 2 hr restraint stress in comparison to T lymphocytes from control mice. We utilized targeted cDNA arrays comprised of 100 genes involved in DNA damage, repair, and apoptosis, to detect differential changes in gene expression. We also used comet assays to directly assess changes in DNA damage. Although we could not detect significant stress-related DNA damage in comet assays, at 30 minutes following restraint, we observed significant increases in expression of genes serving as sensors of DNA damage, including MSH genes and RAD53. Strikingly, expression of GADD45 g, a gene responsible for regulating cell cycle arrest and apoptosis was also significantly up-regulated. We also observed a markedly enhanced expression of PURA, which regulates cell proliferation. These data indicate at the molecular level that restraint stress activates genes that are responsible for priming the T cell to either undergo apoptosis or proliferation.

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Effects of stress on skin immunity and skin cancer

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Stress is believed to suppress immune function and increase susceptibility to infections and cancer. Paradoxically, stress also exacerbates autoimmune and inflammatory diseases including psoriasis and numerous other skin conditions suggesting that stress may have bidirectional effects on immunity. It has recently been shown that while chronic stress generally suppresses immune function, acute stress is immunoenhancing. Acute stress enhances dendritic cell, neutrophil, macrophage, and lymphocyte trafficking, maturation, and function, tapping into pathways that enhance innate and adaptive immunity. Acute stress experienced prior to novel antigen exposure increases memory T cell formation and results in a significant and long-lasting increase in immunity. Acute stress experienced during antigen re-exposure enhances a secondary immune response. This suggests that depending on the condition against which the immune response is directed, stress can enhance the acquisition and expression of immunoprotection or immunopathology. In contrast, chronic stress suppresses or dysregulates innate and adaptive immune responses through mechanisms that involve changes in the Type 1-Type 2 cytokine balance, or suppression of leukocyte numbers, trafficking, and function. Chronic stress increases susceptibility to skin cancer by suppressing Type 1 cytokines and protective T cells while increasing suppressor T cell function. We have suggested that the primary purpose of a physiologic stress response may be to promote survival, with stress hormones and neurotransmitters serving as beacons that prepare the immune system for potential challenges (e.g. wounding or infection) perceived by the brain (e.g. detection of an attacker). However, this same system may exacerbate immunopathology if the enhanced immune response is directed against innocuous or self-antigens, or if the system is over-activated as seen during chronic stress. In view of the ubiquitous nature of stress and its significant effects on immunoprotection and immunopathology, it is important to further elucidate the mechanisms mediating stress-immune interactions and to meaningfully translate findings from bench to bedside.

Stress, Neural Modulation and Cutaneous Immunity II

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Stress-induced immune dysfunction

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It is now well established that psychological stress can induce immune dysregulation and that communication between the central nervous system and the immune system occurs via a complex network of bidirectional signals linking the nervous, endocrine and immune systems. Stress-induced immune dysregulation has been shown to be significant enough to result in health consequences, including reducing the immune response to vaccines, slowing wound healing, reactivating latent herpesviruses, such as Epstein-Barr virus (EBV), and enhancing the risk for more severe infectious disease. Chronic stress/depression can increase the production of proinflammatory cytokines, like IL-6. High serum levels of IL-6 have been linked to risks for several conditions, such as cardiovascular disease, osteoporosis, type 2 diabetes, and some cancers. The association of stress with cancer is still not well understood. The possibility that physiological changes induced by stress could act as a co-factor for malignant disease is being explored.

The cellular immune response plays a major role in the wound healing process. Studies from our laboratory and others have shown that stress can have a significant impact on the early phase of wound healing. The delay in the wound healing process was related to the down-regulation of the production of two proinflammatory cytokines, IL-8 and IL-1α, at the wound site. These cytokines play an important role in the early phase of wound healing. There is also a literature describing the impact of stress on both the antibody and T-cell responses to vaccines including hepatitis-B virus, influenza virus, and pneumococcal bacteria. These studies support the notion that stress-induced changes in the immune response are large enough to have impact on risk for infectious disease and protection associated with getting a vaccination. This relationship is particularly important with respect to the concerns for a future influenza virus pandemic. These studies also have implications for cancer vaccines being developed. The field of psychoneuroimmunology has provided new insights to help understand the pathophysiological processes that are linked to the immune system.

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Neuroendocrine regulation of cutaneous immunity

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The skin is the largest organ of the body and plays a central role in host defense. Disorders of the skin immune activity are implicated in the pathogenesis of cutaneous infections, skin malignancies and acquired inflammatory skin disorders. Immune cells of the skin include epidermal keratinocytes (EKs) which function both as physical barrier and early warning system, Langerhans cells (LCs), intraepithelial lymphocytes, dermal dendritic cells (DCs), mast cells and cutaneous memory T cells. Recently, we showed that the sympathetic nervous system affects DCs function and modulate their migration and Th1 priming ability. Others and we reported that catecholamines may affect skin LCs and bone marrow derived DCs migration and antigen presenting ability via β2-adrenergic receptors (β2-ARs). We also reported that the endogenous endocannabinoid 2-arachydonoylglycerol may recruit DCs in the skin and act as adjuvant for Th1 adaptive response. Besides DCs, EKs are important players in cutaneous immune responses. Both cell types express ARs and pattern-recognition receptors that recognize specific microbial components. We observed that activation of the Toll-like receptor 2 (TLR2) agonist by intradermal injection of S. Aureus peptidoglycan (PGN) along with β-adrenergic receptor (β-AR) inhibition induced an increased expression of inflammatory cytokines and chemokines which in presence of a soluble protein antigen acted as adjuvant for a Th1 response. On the contrary, the β-adrenergic blocking did influence neither the inflammatory nor the adaptive response when the TLR4 agonist LPS or the TLR9 agonist CpG oligodeoxynucleotide were used. The TLR2 boosting involved both β1-ARs and β2-ARs and were principally consistent with the pattern of TLRs expression and response in EKs. As β-ARs signaling defects together with TLRs activation are thought to serve as initiation and/or persistence factors for numerous Th1-sustained inflammatory skin diseases, we might have disclosed part of the relative pathogenetic mechanisms by linking the skin adrenergic system and TLR2 recognition.

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Catecholamines and Langerhans cells

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It has long been postulated that the sympathetic nervous system plays a role in the modulation of immune responses and there is increasing experimental evidence that it directly participates in cutaneous inflammation. Sympathetic fibers travel together with sensory nerves to peripheral tissues and appear as single nerve fibers in dermis and epidermis where they release norepinephrine as their main neurotransmitter. The adrenal medulla contributes all of the circulating epinephrine and some norepinephrine, which can reach the target organs through humoral circulation. Catecholamines can activate a number of target cells within the skin, among them Langerhans cells. Most of the experimental evidence to date indicates a suppressive effect of the sympathetic nervous system on Langerhans cell function, but its effect on cutaneous inflammation in general is still a field of great controversy. The timing of exposure to a stimulus as well as the type and amount of antigen encountered by dendritic cells (DC) is critical to the outcome of the immune response. Administration of a stress hormone or exposure to a stressor before the DC encounters antigen may diminish the immune response towards that antigen, while a stressor may enhance immune function when acting on a maturing DC or before re-exposure to the antigen. Overall, catecholamines appear to contribute to the complex system of neuroendocrine regulation of skin dendritic cells that allows for adaptive responses to various stressors.

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Regulation of cutaneous immune system by odorant inhalation

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Since we detected an association between the epidermal Langerhans cells and nerve fibers, we have tried to understand the skin as an organ closely connected to the whole body. Data suggesting the application of odorants to the skin are introduced.

Inhalation of some odorants decreased the number of Langerhans cells and suppressed allergic contact hypersensitivity reaction. Measurement of serum corticosterone and blocking effects of antagonist suggest the involvement of glucocorticoid in the action of odorants. When another type of odorant was selected by EEG analysis and applied to immobilized mice, downregulation of epidermal Langerhans cells and contact hypersensitivity reaction was prevented. Odorants supposedly affect the skin via the HPA axis but other pathways may also exist. Inhalation of an odorant suppressed the increase in the level of substance P in peripheral blood after interview stress. Mast cell degranulation was also suppressed by odorant, suggesting the possible regulation of itch. The level of anti-aging/stress hormone, DHEA, increased after daily use of the odorant. Other groups also reported the daily use of odorants improved the clinical score of atopic dermatitis.

Stress and Skin Barrier Function

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Overview of the skin barrier

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The primary function of the epidermis is to protect us, i.e., to prevent desiccation and to serve as a barrier to physical, chemical and biological threats in the environment. The skin barrier has 3 main components; 1) the cornified envelopes that provide mechanical strength and a physical wall, 2) the lipids that prevent water loss as well as blocks entry of chemical and biological agents, and 3) a sophisticated set of intracellular signal transduction pathways and extracellular, secreted cytokines, chemokines and growth factors that alert the underlying tissues and initiate biological defenses. The structure of the cornified envelope, elucidated by Peter Steinert and others, consists of several proteins crosslinked by transglutaminases. The proteins include loricrins, involucrin, plakins, small proline rich proteins, keratins and other markers of epidermal differentiation. The epidermal lipids, characterized by Peter Elias and others, mainly consist of free fatty acids, cholesterol and ceramides. The signaling in the epidermis comprises proinflammatory and immunomodulating proteins produced by either keratinocytes, e.g., IL-1, IL-8, TNFα, or other cell types, e.g., IFNγ. Keratinocytes express a large number of receptors on their surface, and can be affected by autocrine signals, i.e., those released by nearby keratinocytes, or by paracrine and endocrine signals from other epidermal cell types, from the dermal component of the skin, including fibroblasts, endothelial cells, neurons, lymphocytes etc. and from the circulation. We have conducted a genomic study of regulation of gene expression in epidermal keratinocytes, focused on the effects of proinflammatory and immunomodulating cytokines. Using a skin equivalents model, i.e., stratified, cornified keratinocytes grown on air-liquid interface, we examined the effects of disrupting the lipid barrier on the gene expression in keratinocytes.

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Effects of acute and chronic stress on skin: Clinical studies

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Psychological stress has long been associated with exacerbation of dermatologic disorders, and recent work has examined the effects of stress on skin physiology. Acute stress is known to activate the autonomic nervous system and the hypothalamic-pituitary adrenal axis, and to activate or suppress different aspects of the immune system, all of which have significant impact on skin function. Chronic stress, however, appears to promote a different pattern of response in these systems. A series of studies have been conducted to compare the effects of acute and chronic stress on skin in healthy humans who do not have skin disorders. Laboratory stressors were used to induce acute stress responses and individuals with post-traumatic stress disorder were used as a model of chronic stress. Both a simulated job interview and sleep deprivation impaired skin barrier function recovery. In a separate study, the job interview stress also suppressed delayed-type hypersensitivity. In contrast, individuals with post-traumatic stress disorder showed enhancement of skin barrier function recovery and delayed-type hypersensitivity. Individuals with post-traumatic stress disorder also had smaller responses to an irritant, sodium lauryl sulfate. These divergent skin response profiles produced by acute laboratory stress and chronic psychological stress point out the need to quantify dose and duration of stress when studying effects of stress on the skin. In addition, individual differences in hormonal responses to stress, which may arise from genetic, developmental, or environmental factors, appear to modulate the effects of stress on skin function.

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Negative effects of psychological stress on epidermis: Role of glucocorticoids

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Psychological stress (PS) exerts well-known adverse cutaneous effects, including exacerbation of inflammatory dermatoses and delayed wound healing, which have been largely attributed to 'neuro-immuno-endocrine' system dysfunction. Since these skin conditions are characterized by abnormalities in permeability barrier function, which in turn, 'drive' downstream metabolic events, including inflammatory responses, we hypothesized that PS could directly compromise barrier homeostasis. We and others then showed that several different types of PS in humans reversibly alter permeability homeostasis, demonstrated further by the normalization of barrier function by co-administration of either tranquilizers (or certain aromatics), in the face of PS in murine analogues of human PS. The PS-induced abnormality in barrier function could be further attributed to inhibition of epidermal proliferation, differentiation, and lipid synthesis. PS was accompanied by increased levels of endogenous glucocorticoids (GC) in the murine models, which are solely responsible for the epidermal functional abnormalities rather than neuro-immuno-endocrine mechanisms, because co-administration of either antalarmine, an inhibitor of GC production, or RU486, an inhibitor of GC peripheral action, completely normalizes barrier function in the face of PS. Finally, short-term administration of either systemic or topical GC provokes dose-dependent epidermal functional abnormalities, which are mechanistically identical to PS; i.e., GC inhibit epidermal proliferation, differentiation, and lipid synthesis.

Because PS/GC profoundly inhibit epidermal lipid synthesis, we assessed whether topical applications of barrier repair lipids; i.e., ceramides, cholesterol, and free fatty acids, in an equimolar ratio, would reverse the PS/GC-induced structural/functional abnormalities. Yet, lipid replacement alone corrected only the barrier abnormality in the face of PS, without normalizing epidermal proliferation or differentiation. Since some activators of the 'liposensor' subclass of class II nuclear hormone receptors, PPARα, γ, β/δ, and LXR, not only stimulate epidermal lipid synthesis, but also regulate epidermal proliferation and differentiation, we next assessed whether these agents could reverse the PS/GC-induced abnormalities. Indeed, with certain liposensor activators (plus the triple lipid mixture), we could normalize epidermal proliferation, differentiation, and lipid synthesis, resulting in full normalization of structure and function, in the face of PS/GC. Hence, co-applications of triple-lipid mixtures of key barrier lipids, along with one or more liposensor activators, could negate the negative effects of GC (and PS) on the epidermis.

Stress, Connective Tissue, and Wound Healing

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Stress and wound healing of the skin

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When the skin is wounded, this large complex organ experiences significant stress. The skin cells at the wound site experience a major Sea Change. First, these cells experience acute hypoxia as the blood vessels are suddenly clotted and the ingress of oxygen-containing red blood cells stop. Second, for the first time, these cells are bathed by serum rather than by plasma. Thirdly, the wound site experiences a sea of pro-inflammatory cytokines and proteolytic enzymes from the degranulation of platelets and the ingress of inflammatory cells. We have evidence that many of these stress signals are Mother Nature's mechanism for re-programming skin cells from a stable quiescent state into a wound healing mode required to heal the skin. Although wound healing is a multi-step complex process, the re-epithelialization step is critical for protecting the host from water loss, salt loss, environmental toxins, infection and sepsis. Re-epithelialization requires keratinocytes to change from a differentiation program into a program of lateral cell migration. Acute hypoxia helps the keratinocytes make that transition by promoting keratinocyte lamellipodia formation, expression of matrix metalloproteinases (MMPs) and enhancement of cell migration. Acute hypoxia invokes a transcription factor called hypoxia inducible factor 1 (HIF-1) which is associated with increase expression and secretion of a heat shock protein, HSP-90 alpha. The exogenous addition of HSP-90 alpha to normoxic keratinocyte motility assays duplicates the enhanced cell migration generated by hypoxia. Acute wounding of skin, likely also invokes systemic signals throughout the host's body. Evidence for this is that when we inject molecularly engineered human skin cells that over express type VII (anchoring fibril) collagen into the tail vein of a wounded mouse, the cells selectively home to the wound site, secrete type VII collagen into the wound, and promote wound healing. The exogenously delivered human type VII collagen incorporates into the mouse's healing skin and forms human anchoring fibrils. Cells and type VII collagen are not found in other organs or in un-wounded skin. These experiments show the cross-species plasticity of wound healing biology and strongly suggest some unknown mechanism by which acute wounds signal distant cells to home to the wound and participate in wound healing.

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Stress and wound healing

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Several studies from our laboratory have addressed the influence of psychological stress on wound healing, as well as local and systemic proinflammatory cytokine production. For example, women caregiving for a relative with Alzheimer's disease took 24% longer than well-matched controls to heal a small, standardized dermal wound. We hypothesized that higher levels of psychological stress would be associated with impairments in the local inflammatory response. Skin blister wounds were used because they allowed us to monitor the kinetics of this key phase *in vivo*. Couples were admitted twice to a hospital research unit for 24 hours in a crossover trial. During the first admission couples had a structured social support interaction, and during the second they discussed a marital disagreement. Wound healing was assessed daily following discharge. Couples' blister wounds healed more slowly, and cytokine production (interleukin-6, tumor neucrosis factor-α, and interleukin-1β) was also lower at blister wound sites following marital conflicts than after social support interactions. Moreover, couples who demonstrated consistently higher levels of hostile behaviors across both their interactions healed at 60% of the rate of low hostile couples. These data provide further mechanistic evidence of the sensitivity of wound healing to everyday stressors.

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Experimental models of stress and wound healing

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Psychological stress has been shown to delay wound closure in humans and animals. The stress of academic examinations or caregiving for Alzheimer's patients has been shown to delay wound closure by 40% and 24%, respectively (Marucha *et al*. 1998; Kiecolt-Glaser, *et al*. 1995). In a mouse model, restraint stress has been shown to delay wound closure by 27%. The mechanisms by which stress modulates closure during wound healing remain to be fully elucidated. Two major pathways appear to be involved, the hypothalamic pituitary adrenal (HPA) axis and the sympathetic nervous system (SNS). HPA axis activation results in adrenal production of glucocorticoids and epinephrine. Glucocorticoids can regulate the production of proinflammatory cytokines and chemokines required for phagocyte recruitment and activation, as well as growth factors required for tissue repair. Indeed, mice subjected to restraint stress have lower levels of proinflammatory cytokines and growth factors in their wounds, impaired angiogenesis, and increased rates of infection. One day after wounding, levels of IL-1β and VEGF are significantly reduced in stressed mice. Treatment of stressed mice with RU486, a glucocorticoid antagonist, restores normal expression of IL-1β and partially ameliorates normal healing. The other important pathway, the SNS, can cause peripheral vasoconstriction, impairing blood flow to the wound, and resulting in tissue hypoxia. This limits oxygen availability for tissue repair and resistance to infection. Using novel technology, electron paramagnetic resonance, we found that the pO2 in wound tissue of stressed animals is 20% lower than in controls. Additionally, oxygen modulated genes are dysregulated by stress in wounds. For example, iNOS gene expression is up-regulated three fold in wound tissue from stressed mice. Treatment of stressed mice with hyperbaric oxygen (HBO) restores normal healing and normal iNOS gene expression. Thus, our findings suggest both the HPA axis and the SNS are important pathways activated by stress that result in impaired healing.

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Stress and skin aging

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Stress has long been believed to accelerate aging processes throughout the body, but only recently have mechanistic insights been available. One relevant line of research concerns telomeres, tandem repeats of TTAGGG and its complement that cap all mammalian chromosomes. Telomeres progressively shorten with each round of cell division, and critical telomere shortening causes cells to permanently cease dividing, a state termed senescence and believed to represent the cellular basis of organismal aging. Acute oxidative stress greatly accelerates telomere shortening and entry into senescence, and low-grade oxidative stress due to aerobic metabolism is through to be a major contributor to aging. Our group has found that telomere homolog oligonucleotides (termed T-oligos), when provided exogenously to cultured cells, rapidly concentrate in the nucleus and at low doses and/or brief exposures enhance the cell's innate defense mechanisms, but at high doses and/or protracted exposures may precipitate senescence. Using T-oligos as a probe, it is thus possible to document at the cellular level the phenomenon of hormesis, improved physiologic function and resistance to environment insults that follow low levels of stress, and a continuum of responses at higher levels of stress that include premature aging. Extensive data suggest that T-oligos mimic telomere disruption with exposure of the single stranded TTAGGG repeats that constitute the final 100–400 bases of the telomere 3′ strand, and these data therefore strongly implicate DNA damage and the telomere in both chronologic aging and stress-induced accelerated aging.

Stress and Skin Disorders

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Stress and acne / rosacea

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There increasing evidence that neurogenic mediators contribute - with or without UV irradiation - to skin inflammation. Among common skin diseases acne, the most common skin disease, and rosacea are characteristic examples of inflammatory disorders initiated in or involving the facial sebaceous follicles. Intrinsic and environmental factors have been accused to trigger follicular inflammation. In the last few years, increasing evidence has been provided that the pathways involved in these processes include neuropeptides. Interestingly, the sebaceous glands exhibits an independent peripheral endocrine function and expresses receptors for neuropeptides. A complete corticotropin releasing hormone (CRH) system has been detected by immunohistochemical and molecular biological means in human sebocytes *in vivo* and *in vitro*. The capability of hypothalamic CRH to induce lipid synthesis, steroidogenesis and interact with testosterone and growth hormone indicates its involvement in the clinical development of acne, especially since expression of the CRH system molecules is abundant in acne-involved skin. On the other hand, rosacea, a chronic skin disorder affecting primarily the convexities of the central face, has been attributed to vasculature alterations, chronic excessive sun exposure, matrix degeneration, hormonal influence, but also to emotional stress. Own histologic findings present strong evidence that almost all variants of rosacea start as an actinic lymphatic vasculopathy, thereby they corroborate Kligman's postulate that rosacea should be viewed as a UV-induced dermatosis. Further molecular and cellular biology experiments lead us to propose an aggravating role of CRH on UV light-induced solar elastosis, vessel dilatation, and immunosuppression in rosacea.

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Stress and psoriasis

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Psoriasis is a common immune-mediated disease that occurs in approximately 2% of the population. Its aetiology is unknown but there is a strong immuno-genetic component to this disease and a number of environmental triggers, including stress. Approximately 60% of patients report that stressful life events may either trigger and/or exacerbate their condition. Furthermore the major stressor in patients' lives is avoidance coping. We have shown that psoriasis produces significant psychosocial disability which does not correlate with physical severity. Patients who are high/pathological worry are less likely to clear with PUVA therapy than patients who are in the normal/low worry category. Cognitive behavioural therapy as an adjunct to traditional therapies for psoriasis significantly improves response. Patients with psoriasis have a relatively hypo-cortisolic response to an experimental stressor – 'Trier'. This is particularly marked in patients whose psoriasis is stress-reactive. The role of Langerhans' cells and neuropeptides in this abnormal response to stress is under investigation as are the roles of corticotrophin releasing hormone (CRH) and CGRP. It is likely that there will be significant parallels between stress triggering of psoriasis and triggering of other inflammatory diseases such as arthritis, colitis, atopic dermatitis and acne.

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Role of psychological stress in the expression of allergic and inflammatory dermatoses

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Psychological stress has been associated with disturbed regulation of key physiological systems (e.g., neuroendocrine and immune processes, autonomic nervous system functioning, and oxidative stress pathways) which may in turn influence inflammatory and allergic dermatoses. Hormones and neuropeptides released into the circulation when individuals experience stress are thought to be involved in regulating both immune-mediated and neurogenic inflammatory processes in the skin. Dysregulation of normal homeostatic processes can occur in the face of chronic stress leading to chronic hyperarousal and/or hyporesponsiveness that may impact disease expression. Dysregulation of normal homeostatic neural, endocrine, and immunologic mechanisms can occur in the face of chronic stress leading to chronic hyperarousal and/or hyporesponsiveness that may impact disease expression. A growing number of stress mediators including glucocorticoids, corticotrophin-releasing hormone (CRH), nerve growth factor, neurotensin, substance P and cytokines have been implicated in both animal and human research. Mechanisms linking psychological stress to specific skin disorders, including atopic dermatitis, psoriasis, and urticaria, will be reviewed to highlight our growing understanding of the 'brain-skin connection'. The skin is arguably the most accessible organ system for the continued study of stress mediators which promises to inform therapeutic interventions for both dermatologic disorders and more systemic diseases.

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The role of stress in the initiation and progression of cancer

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The conclusion of an earlier review of seventy longitudinal studies (published between 1978 and 2002) on the role of psychological factors in the initiation and progression was, that there is no psychological factor whatsoever for which such an influence has been convincingly demonstrated in a series of studies. The majority of the studies did find a relationship between psychological factors and disease initiation and progression, but rarely for the same factor (Garssen, 2004).
This presentation is restricted to the role of stress. Stress will be operationalized as having experienced serious life events in general or bereavement and other loss events in particular, experiencing negative emotional states (distress, anxiety, depression), having (had) a psychiatric diagnosis, especially a depressive disorder, or the tendency towards helplessness. Helplessness was the only stress factor for which some support for an effect on cancer development was found in the earlier review. It will be discussed whether recent studies have changed this conclusion. Several methodological shortcomings will be discussed as possible reasons for not finding expected relationships. The most important one seems to be not having investigated the interactive effect of several psychological factors, and of psychological and biomedical risk factors.
Garssen B. Psychological factors and cancer development: Evidence after 30 years of research. Clin Psychol Rev 24, 2004, 315–338

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"Hairy" matters in the stress circus: The hair follicle as a unique model for stress research

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While it has long been claimed that psychoemotional stress may trigger hair loss (e.g., stress-induced telogen effluvium, alopecia areata), sound evidence that stress impacts negatively on hair growth had been missing until recently. Using a mouse model for chronic stress (sonic stress), our collaborators (P Arck and E Peters, Berlin) and we could recently show that sonic stress indeed exerts profoundly inhibitory effects on murine hair growth (e.g., inhibition of proliferation, stimulation of apoptosis of hair keratinocytes, premature catagen development, stimulation of perifollicular inflammatory cell infiltrates and mast cell degranulation). These effects are critically dependent on substance P (SP) and its receptor (NK1), with NGF as likely up-stream regulator, and mast cells as down-stream effector cells. Surprisingly, in mice, these hair growth-inhibitory effects are significantly mitigated by topical minoxidil.

Intriguingly, the hair follicle, both in mice and man, is not only an exquisitely sensitive target organ for essentially all major mediators of system stress responses (e.g. SP, NGF, catecholamines, prolactin, cortisol, ACTH, CRH), since it expresses cognate receptors, but also generates these stress mediators locally. Most notably, we have recently shown that human scalp hair follicles even have established a fully functional peripheral equivalent of the central HPA stress response axis, including the synthesis of cortisol and regulatory feedback loops that are characteristic for the central HPA stress response axis.

Research into the functional roles of such intrafollicularly generated "stress hormones" is still in its infancy. However, there are first indications that some of these locally generated (neuro-)endo-crine signals are involved in maintaining the immune privilege of the anagen hair bulb (most notably, alpha-MSH and cortisol), while others may primarily have proinflammatory, pigmentation- and/or growth-modulatory effects. In any case, as a prototypic neuroectodermal-mesodermal interaction unit, the hair follicle is both a target and a source of stress mediators. This makes it a uniquely instructive general model for basic and clinically relevant stress research, and for dissecting how systemic and peripheral stress response systems communicate and are coordinated.

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Stress and urticaria

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Psychological stress is recognized as a clinically important factor in urticaria however there are no recently reported studies on the prevalence of stress in this patient group. Emotional stress has been implicated in chronic urticaria and angioedema including hereditary angioedema associated with C1 esterase inhibitor dysfunction; and in cases of acute urticaria especially adrenergic urticaria[1] and some cases of cholinergic urticaria.[2,3] The underlying cause of urticaria is typically not determined in around 50% of patients and in earlier studies stress has been implicated as the primary underlying factor in 11.5%[4] to 21%[5] of patients and a contributory factor among 24%[4] to 68%[5] of patients. 'Increased mental tension and fatigue' was spontaneously reported as a factor by 77% of 43 patients studies[6]; and stressful life situations were associated with the onset of symptoms in 51% of 100 patients with chronic urticaria or angioedema *versus* 8% of surgical controls.[5] In a study of 48 patients exacerbation by emotional stress occurred in 77% of patients with cholinergic urticaria[2], 82% with dermographism2 and no patients with cold urticaria. Severe emotional stress may exacerbate urticaria regardless of the cause. Catastrophic life events such as earthquakes have been associated with urticaria; and severe psychological trauma and posttraumatic stress disorder have been associated with urticaria and angioedema which sometimes appears to recur in the same body region where the initial trauma was inflicted e.g., wheals where the patient had been strapped as a child or angioedema of the mouth associated with flashbacks of oral sexual abuse.[7] Various pathophysiologic mechanisms may play a role: earlier studies[8] have shown that under psychologic stress the threshold for reactive hyperemia decreases; mast cell degranulation may be triggered by neuropeptides such as corticotrophin releasing hormone (CRH)[9] which is synthesized by the human skin, and acute immobilization stress[10] in rats has been shown to trigger mast cell degranulation and increased vascular permeability, an action which was dependent on CRH and Substance P. References: 1. Shelley WB *et al*. Lancet 1985; 2. Czubalski *et al*. Dermatologica 1977; 154: 1–4; 3.Grattan CEH *et al*. J Am Acad Dermatol 2002; 46: 645–657; 4. Champion RH *et al*. Br J Dermatol 1969; 81:588–597; 5. Rees L. J Psychosom Res 1957; 2: 172–198 ; 6. Michaelsson G. Acta Derm Venereol(Stockh) 1969; 49: 404–416; 2: 1031–1033; 7. Gupta MA *et al*. Dermatologic Clin 2005; 23(4):649–56 ; 8. Graham DT *et al*. JAMA 1950; 143: 1396–1402; 9. Papadopoulou N *et al*. J Invest Dermatol 2005; 125: 952–955; 10. Singh LK *et al*. Brain Behav Immunity 1999; 13: 225–239.

Abstracts for Poster Presentation

Stress, Neural Modulation and Cutaneous Immunity

1

Beyond the Cartesian Paradigm:A nonlinear and synergetic approach to psychoneurodermatology and psychoneuroimmunology

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Our poster proposes a new way of conceptualizing how stress impacts on immune system functioning and thus effects the skin. This post modern approach is based on findings in theoretical physics, like Chaos Theory and Synergetics. It allows for the development of a nonlinear, fractal and holistic model that offers a much more comprehensive and elegant means of mapping the complex neurobiological, neuroimmunological and psychological processes that are involved skin disease. Research indicates that many, if not all, biological and neurobiological activities obey non linear and fractal principles of a synergistic approach. Yet the description of the ways in which the immune system functions under stress can still be linear and reductionistic, confined within an anachronistic Cartesian paradigm. For example, rather than proposing that cortisol and its antagonistic DHEA are secreted or suppressed according to the orders of the HPA axis, in the post modern approach cortisol and DHEA account for a dynamic iterative and nonlinear process while the HPA axis acts as a control parameter, a holistic part of a synergistic subsystem within other self-similar subsystems. Adopting a post modern theory allows for more accurate descriptions of the complex, mutually influencing feedback loops that regulate the skin's response to stress. Moreover, this approach provides a theoretical underpinning for the relationship between the psyche and the soma that goes beyond the mind body dualism that still imprisons much of our work. In the post modern approach psyche and soma are two sides of the same coin, each part has its own 'matter' and cannot be derived reductionistically from its other part. Through the use of clinical case examples, this poster will elaborate upon these ideas and illustrate the superior heuristic depth of this comprehensive system approach.

2

Epidermal Langerhans' cell frequency is modulated by acute social stress

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In response to pathogenic or environmental stimuli epidermal Langerhans' cells (LC) are stimulated to migrate from the skin to draining lymph nodes. Previous studies in mice have shown that acute restraint stress is capable of modulating LC frequency and function. We have now studied the effects of acute psychological stress on LC frequencies in normal human epidermis. One 6 mm punch biopsy was taken under local anaesthesia from sun-protected buttock skin of twenty six healthy volunteers (15 male, 11 female; mean age: 22.6 +/−3.8 yrs; range: 18–36 yrs). Fifteen of the volunteers were subjected to the Trier social stressor in the form of assessed public speaking. A second 6 mm biopsy was taken from the contralateral buttock either 4 h (n = 5) or 24 h (n = 10) later. A control group (n = 11) was biopsied after 24 h but not subjected to the stressor. Epidermal sheets were prepared and stained for CD1a expression to determine changes in LC frequency. No significant changes in epidermal LC frequency were observed at 4 h after exposure to the stressor. However, 24 h after the stressor, LC numbers showed a significant 14.02 +/−9.5% mean reduction compared with baseline values. This reduction is similar to that induced by other stimuli for LC migration such as the cytokines tumour necrosis factor-α and interleukin-1β (20–30%) and represents the proportion of LC responsive to such signals. In control subjects there was no significant change in LC frequencies after 24 h. Our data suggest that acute interview stress is capable of modulating LC frequency in naïve healthy skin. The mechanisms through which stress modulates skin immunity are currently unknown but the association between stress and LC may give new insights into how skin inflammation is triggered or aggravated by stressful life events.

3

Signaling via NK-1R activates skin dendritic cells and favors Th1 responses following genetic vaccination

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The skin represents an ideal site for immunizations which require induction of effector CD4 Th1 cells and CD8 CTL. The immunogenicity of the skin correlates with the high number and plasticity of epidermal and dermal dendritic cells (DC), which are sensitive to pro-inflammatory changes in the microenvironment. Genetic immunization with the gene gun (GG) results in expression of transgenic (tg) antigen (Ag) by skin resident DC followed by presentation to CD4 T cells and CTL. However, it is not clear whether the GG induces Th1 responses. The pro-inflammatory neuropeptide substance P (SP) secreted in response to danger signals fulfills the requirements for a Th1 biasing adjuvant through the interaction of SP with the neurokinin 1 receptor (NK-1R). Here we tested whether local injection of the NK-1R agonist (NK-1Ra) [$Sar^{9}Met\ (O2)^{11}$-Substance-P] favors Th1 biased immune responses without decreasing the efficiency of tg Ag expression following GG immunization. We utilized the GG to deliver the luciferase reporter (pCMV-Luc) or the Ag ovalbumin (pCMV-OVA), in the presence or in the absence of NK-1Ra, to the skin of C57BL/6 mice and analyzed tg expression and T cell stimulatory activity of skin DC *in vivo*. NK-1Ra augmented Luc expression and activation of DC that traffic to skin DLN. These effects were due to an increased activation state of skin DC as determined by increased levels of nuclear translocation of NF-kB and by high expression levels of IA^{b} and CD86 on skin derived DC which have migrated to the DLN. *In vivo* killing assays showed efficient induction of OVA specific CTL by GG and NK-1Ra + GG. Importantly, compared to GG alone, NK-1Ra + GG induced a higher secretion of IFN-γ from CD4 and CD8 T cells and improved DTH responses. These effects were abrogated by injection of specific NK-1R antagonist. Our results demonstrate that NK-1Ra has an adjuvant effect able to promote and sustain effector Th1 biased cellular immune responses following skin genetic immunization.

4

The impact of acute social stress (modified Trier paradigm) on calcitonin gene-related peptide.

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It is widely recognised that psychological stress may have a negative impact on skin disease but the underlying mechanisms are poorly understood. We investigated the response of cutaneous innervation [protein gene product (PGP 9.5)] and the neuropeptide, calcitonin gene-related peptide (CGRP) to social stress (modified Trier paradigm). CGRP has diverse effects on immune cells and is an important mediator of neurogenic inflammation. PGP 9.5, a cytoplasmic protein, is a general marker of neurones.

On day 1, a 6 mm punch biopsy was taken from sun-protected buttock skin of all participants [n = 27:15 male, 12 female; mean age:22.4(18–36 yrs)]. The second biopsy was taken from the contralateral buttock 4hrs (n = 5) or 24 hrs (n = 10) after the social stressor. Controls (n = 12) did not take the Trier test and biopsies were taken 24 hours apart. The pattern and quantity of sensory nerve fibres and CGRP were determined by immunolocalisation (CGRP- and PGP 9.5-antibodies), immunofluorescent microscopy and the Image Pro-Plus package®. We observed an increase in PGP 9.5+ fibre density (4 hrs: 101%, p = 0.078; 24 hrs: 56%, p = 0.08) and a decrease of CGRP+ fibre density (4hrs: 35%, p = 0.19; 24 hrs: 39%, p = 0.054). By contrast, non-stress controls at 24 hrs showed a 37% decrease (p = 0.03) in PGP 9.5+ fibres and a 15% increase (p = 0.16) of CGRP+ fibres. These data indicate that CGRP secretion is triggered within 4 hours of exposure to an acute social stressor; increased detection of PGP 9.5+ fibres is consistent with recruitment of neuropeptides. Our results suggest that acute social stress could affect the modulation and release of neuropeptides such as CGRP and this may be important for our understanding of the 'brain-skin' axis.

5

Enhancing cutaneous neuronal metabolism

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The role of the epidermal neural network in maintaining the general health and well being of the skin is becoming more widely appreciated [1–3]. Recent investigations have shown that as a person ages, the density and function of epidermal innervations decreases resulting in changes in the skin's ability to handle general daily stresses such as thermoregulation, sensory perception, and inflammation and immune response. These changes are also dependent on a variety of extrinsic factors such as solar exposure, environmental pollution and temperature among others. While increasing the number of epidermal nerve branches might be one potential way to improve the skin's nerve cell functions, another way would be to improve the ability of the existing nerves to work better. Using an *in vitro* testing model, dorsal root ganglia were treated with various test products to see if cellular metabolism could be enhanced, Two test products, 1% of an aqueous extract of *Rhodiola rosea* (active content, 0.01%), previously shown to enhance oxygen respiration in human white adipocytes and a 1% aqueous extract of lysed *Sacchaormyces cerevesiae* (active content 0.25%) were shown to increase nerve cell oxygen consumption compared to PBS and 1% creatine and statistically comparable to 10 micromoles of isoproterenol.

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6

An *in vitro* model for studies on stress and the skin?

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The effects of stress on the skin are mediated by an endocrine network but also probably by the interactions between the peripheral nervous system and the skin. In the epidermis, nerve endings are connected to epidermal cells through synapse-like structures. We have performed a co-culture of epidermal cells and neurons in order to propose an *in vitro* model that could be used for studies on stress and the skin. We used culture dishes with separated domains and microsystems. After 15 days of culture, we obtained a co-culture with respectively equivalents of epidermis, root ganglia and spinal cord. Nerve fibers specifically grew to epidermal cells and to the spinal cord equivalent. By CSLM, we observed synapse-like contacts between nerve endings and Merkel cells or keratinocytes. Electron microscopy confirmed the presence of connections between neurons and keratinocytes. Electrophysiological studies showed that these connections were functional.

Such a model is the first one for studies on interactions between skin and the nervous system. After activation of neurons by electrical current or by mediators such as catecholamines in the root ganglia or spinal cord compartments, this model could be used as an *in vitro* model for studies on stress and the skin.

7

Norepinephrine inhibits Langerhans cell activation by inhibiting cytokine release

E Goyarts, M Matsui, D Maes and T Mammone *Estee Lauder Research Laboratories, (Melville, New York – USA)*

Psychological stress is potentially harmful to the skin because the nervous system responds by releasing norepinephrine, which may suppress cellular immunity by reducing the ability of Langerhans cells (LCs) to be activated. We investigated the effect of norepinephrine on human LC activation. LC activation was evaluated by the release of LPS-inducible cytokines into the culture supernatant. Human bone-marrow-derived dendritic cells, containing 40% plasmacytoid dendritic cells and 60% myeloid dendritic cells, were obtained from MatTEK. These cells were grown in a patent-pending media. Dendritic cells were stimulated by LPS in the presence or absence of norepinephrine. Protein levels of the LPS-inducible markers, TNF alpha and IL-12 p40, were down-regulated in the presence of norepinephrine. The LPS-inducible markers, IL10 and IL1 beta were also investigated. The specificity of the response was evaluated with alpha and beta adrenergic receptor antagonists. The beta adrenergic receptor antagonists, propranolol and ICI-118,551, increased the LPS-inducible markers, TNF alpha and IL-12 p40, in the presence of norepinephrine and LPS. The alpha adrenergic receptor antagonist, yohimbine, had no affect. Beta adrenergic receptors expressed on the surface of human LC mediate the decrease in immune function. A beta adrenergic receptor antagonist is likely to reverse stress-induced immunosuppression by directly inhibiting norepinephrine engagement of the beta adrenergic receptor.

8

Neprilysin/angiotensin-converting enzyme double-deficient mice: a mouse model to study inflammatory skin disease.

M Fastrich, L Fabritz, TA Luger and TE Scholzen *Ludwig-Boltzmann Institute, Department of Dermatology; Laboratory for Cardiology and Angiology, Medical Clinic, University of Münster, (Münster, Germany)*

The tissue steady-state contents of the vasoactive and neuropeptides bradykinin (BK), angiotensin or substance P and their respective metabolites plays an important role in the regulation of systemic cardiovascular functions as well as in the local control of inflammatory processes. Neprilysin (NEP, CD10) and angiotensin-converting enzyme (ACE; CD143) are key enzymes in metabolizing these peptides and their role in renal and cardiovascular disorders as well as in inflammatory skin disease is widely appreciated. Inhibition of NEP and ACE by combined NEP/ACE (vasopeptidase) inhibitors is beneficial for the treatment of hypertension and cardiovascular disease; however, side effects including an increased risk of angioedema limit their application. By breeding C57BL/6J-NEP−/− and C57Bl/6J-ACE-deficient mice, we have recently generated NEPxACE double deficient mice. Despite a slightly higher mortality rate of the resulting offspring prior to weaning, the resulting pups appear phenotypical normal. Initial characterization of NEP−/−xACE+/− mice reveals a slightly increased body mass (+17%), heart weight (+22%) and masses of left and right ventricle in comparison to wild type animals. In a mouse model for DNFB-induced cutaneous allergic inflammation, sensitized heterozygous NEP+/−xACE+/− mice displayed significantly increased allergic inflammation (ear swelling: +96 – +112%) over 72 h after challenge in comparison to wt mice, which was similar to that observed for homozygous NEP-deficient mice. In the NEPxACE mice, inflammation could be markedly reduced by systemic treatment with a BK B2 receptor antagonist (RA) before sensitization (−34.8 %), whereas reduction by a neurokinin 1 RA was less effective (−9%). These preliminary data indicate that NEPxACE double deficient mice are a promising mouse model to investigate exaggerated skin inflammation meditated by vasoactive peptides including BK and SP.

9

Anti-inflammatory and anti-nociceptic activity in skin by indole quinazoline alkaloids from traditional Chinese medicine

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Evodia rutaecarpa, a small berry fruit unique to China, has been reported to have anti-inflammatory activity. We have previously shown that fractions of Evodia fruit extract, containing indole quinazoline alkaloids (primarily rutaecarpine and evodiamine), were potent inhibitors of UV-induced PGE2 production in cultured human keratinocytes. Due to the variability of anti-inflammatory activity seen with different lots of natural extract we developed a biomimetic mixture of the active components of Evodia fruit extract. Methyl nicotinate (MN) produces a localized erythema within 30 minutes after topical application to human skin. We applied this model of micro-inflammation in 7 normal healthy volunteers (24–59 yr) in order to evaluate the *in vivo* applications of the Evodia biomimetic. A 1% lotion of the biomimetic Evodia extract significantly inhibited MN-induced erythema when applied 60 minutes before, or within 5 minutes after MN exposure, compared to the vehicle control. Lower concentrations of the biomimetic extract applied twice daily for 7–14 days also significantly inhibited erythema after a MN challenge. The biomimetic Evodia extract caused no irritation or sensitization among 50 normal volunteers and resulted in 30% less erythema than bisabolol, the active component of chamomile. Finally, we assessed the anti-nociceptic ability of the Evodia biomimetic lotion using a neurometer which measures the lowest perceptible electrical stimulation of skin. After 30 minutes of topical treatment with Evodia biomimetic, subjects showed an increase in the threshold of skin sensation. This increased threshold correlates to a reduced sensitivity to hot, cold, and mechanical stimuli. These results demonstrate that this Evodia biomimetic has anti-inflammatory and anti-nociceptic activity when applied topically to human skin.

10

Intermedin: A novel skin peptide that is down regulated in atopic dermatitis

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Introduction: Intermedin (IMD), also called adrenomedullin (ADM) 2, is a novel peptide that belongs to the calcitonin/calcitonin gene related peptide (CGRP)/amylin peptide family. Hence, peptides of the CGRP/ADM peptide family have deep impact on skin function and integrity. The function and localisation of IMD, the most recently discovered member of this family, in inflammatory diseases of the skin remained to be determined. Thus, we investigated the significance of IMD in atopic dermatitis (AD) and healthy skin.
Methods: Here, we analyzed the expression of the IMD peptide in human skin of healthy controls, in biopsies from lesional and non-lesional areas of atopic dermatitis skin, and in the immortalized human keratinocyte cell line HaCaT at the transcriptional (quantitative RT-PCR) and translational (immunohistochemistry) level.
Results: Intermedin mRNA and protein could be detected in HaCaT cells and human skin. In addition to keratinocytes, nerve fibres, periglandular cells, arterial/arteriolar smooth muscle cells and pericytes of dermal microvessels were intensely IMD-immunoreactive. The cutaneous expression of IMD-mRNA differed between control persons and AD patients in that the message for intermedin was, compared to healthy skin, significantly reduced in lesional and non-lesional areas of AD skin. This was accompanied by a reduction of intermedin-immunoreactivity in pericytes of the upper dermis.
Conclusion: The results indicate that skin from AD patients is generally affected, and down-regulation of IMD in AD skin is not a secondary phenomenon caused by acute inflammation but is a general characteristic of AD skin. These data further point to a role of IMD expressed by pericytes in conferring higher susceptibility of the skin of AD patients to inflammatory stimuli.

11

Cultured human keratinocytes are able to express functional galanin receptor

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Galanin (GAL) is a biologically active neuropeptide that is widely distributed in the nervous system. GAL exerts diverse action via the GAL receptors (GALR1, GALR2 and GALR3), which belong in the superfamily of G-protein-coupled transmembrane receptors. In human skin, GAL-like immunoreactivity has been reported in free nerve endings and fibers of the dermis, and extraneuronal expression of GAL has also been demonstrated. Although the GAL receptors are essential for the biological function, their expressions in cultured human keratinocytes and in normal skin have not yet been investigated. The aim of our study was to investigate the mRNA and protein expressions of different GAL receptors in cultured human keratinocytes. When reverse transcription PCR was used with different GAL receptor-specific primers, only GALR2 mRNA was identified in keratinocytes. Sequencing of the PCR products proved the presence of GALR2 mRNA in the keratinocytes. The presence of GALR2 protein was next investigated, using a polyclonal antibody against human GALR2. Cultured keratinocytes displayed specific immunohistochemical staining, with higher intensity on their surface. Immunohistochemical investigations of normal human skin specimens revealed that GALR2 was expressed with high intensity in the basal layer of the epidermis and also around the hair follicles in the dermis. GAL treatment of the keratinocytes resulted in an increase in cytosolic $Ca2+$, suggesting that GALR2 is a functional receptor. Further studies are necessary to clarify the biological effects of GAL in the skin.

Stress and Skin Barrier Function

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Connexin 26 regulates epidermal barrier, wound remodeling and promotes psoriasiform response

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Inflammatory skin disorders result in significant epidermal changes, including keratinocyte hyperproliferation, incomplete differentiation and impaired barrier. Here we test whether, conversely, an impaired epidermal barrier can promote an inflammatory response. Mice lacking the transcription factor Kruppel-like factor 4 (Klf4) have a severe defect in epidermal barrier acquisition. Transcription profiling of Klf4–/– newborn skin revealed similar changes in gene expression to involved psoriatic plaques, including a significant upregulation of the gap junction protein connexin (Cx) 26. Ectopic expression of Cx26 from the epidermal specific Involucrin (INV) promoter (INV-Cx26) demonstrates that down-regulation of Cx26 is required for barrier acquisition during development. As juveniles and adults, persistent Cx26 expression keeps wounded epidermis in a hyperproliferative state, blocks the transition to remodeling, and leads to an infiltration of immune cells. Mechanistically, ectopic expression of Cx26 in keratinocytes results in increased ATP release, which delays epidermal barrier recovery and promotes an inflammatory response in resident immune cells. These results provide a molecular link between barrier acquisition in utero and epidermal remodeling post wounding. More generally, these studies suggest that the most effective treatments for inflammatory skin disorders might concomitantly suppress the immune response and enhance epidermal differentiation to restore the barrier.

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Impact of psychological stress on skin barrier

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The human organism is in a state of dynamic equilibrium. The stress system is activated when homeostasis is challenged by extrinsic or intrinsic forces; the stressors. Psychological stress is associated with significant increases in a variety of psychological and physical disorders. The effect of stress on the immune system is well documented and skin disorders have been reported to exacerbate during stressful situations. This study was designed to observe the effects of psychological stress on skin barrier functions and repair. A panel of healthy females in the process of marital separation were tested for skin barrier functions and repair. The panel was chosen on the basis of the intensity of self assessed stress. The control group was an age-matched panel of self assessed ''low-stress'' subjects.

Results imply that there was no correlation between the degree of stress and barrier strength. However, individuals with high stress repaired slower than the individuals with low stress after 3 hours ($R = 0.64$) and 24 hours ($R = 0.74$). In conclusion psychological stress of marital dissolution does not appear to change skin barrier strength but has a negative impact on skin barrier repair functions.

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Measurement of skin surface cortisol and biomarkers in preterm infants

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Background: Epidermal innate immunity is a complex process involving a balance of pro- and anti-inflammatory cytokines, structural proteins, and specific antigen presenting cells in the epidermis. Orchestration of these factors occurs against a background of neuroendocrine/local tissue modulators such as cortisol. Herein we use a new noninvasive sampling method to simultaneously determine multiple factors critical for the development of epidermal innate immune function. Objective: To measure and determine levels of antimicrobial peptides, structural proteins, and cortisol on the skin surface of adults, preterm and term infants. Design/Methods: 61 subjects were studied: 20 adults, 21 premature (< 32 wks, <7 postnatal days) and 20 term infants. A previously described tape stripping method was used for non-invasive sampling of the skin surface, followed by Luminex® Multiplex assay for simultaneous measurement of cytokines interleukin-1 (IL-1), IL-6, IL-8; IL-1 receptor antagonist (IL-1ra), and other biomarkers such as IFN-γ, cortisol, serum albumin, and key structural proteins such as keratins-1, 6, 10, 11, involucrin and fibronectin. Results: In premature infants, there was a significant increase in pro-inflammatory cytokines IL-6, IL-1, and IL-8 compared with samples from term infants and adults. Skin cortisol was significantly elevated in premature infants. Keratin proteins were decreased and involucrin was increased in both infant groups compared to adults. Conclusions: Preterm infants have a unique profile of cytokines (increased IL-6, IL-1, and IL-8) and structural proteins (decreased keratins-1, 6, 10, 11, increased involucrin) on the skin surface. Preterm infants show significant elevations of skin surface cortisol and serum albumin compared to term infants and adults.

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Psychological stress inhibits epidermal antimicrobial peptide production in a glucocorticoid-dependent fashion

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The adverse effects of psychological stress (PS) on epidermal structure and function can be attributed largely to increased levels of glucocorticoids (GC), and systemic/topical GC mimic the effects of PS. PS/GC's negative effects were further linked to profound inhibition of both epidermal lipid synthesis and lamellar body (LB) production/secretion (JID 120:456, 2003; JID124:587, 2005). Since delivery of proteins to nascent LB is dependent upon concurrent lipid deposition (Tissue & Cell 31:489, 1999), we hypothesized that PS/GC could also inhibit the delivery of two LB-delivered, antimicrobial peptides (AMP), mBD3 (murine homologue of hBD2) and LL-37 (carboxyterminal peptide product of CRAMP [murine homologue of hCAP18]), thereby reducing antimicrobial defense during PS/GC. Insomnia-induced PS and both systemic/topical GC down-regulated epidermal mBD3 and LL-37 expression in hairless mice (shown by rt-PCR and immunoflorescence) in parallel with reduced LB production, epidermal lipid synthesis, and impaired permeability barrier recovery after acute abrogations. Blockade of either GC production (by the CRH inhibitor, antalarmin) or GC peripheral action (by Ru486) normalized AMP levels during PS. Blockade of endogenous GC also increased expression of these peptides in normal murine epidermis, suggesting that physiologic levels of GC suppress AMP. Finally, topical co-administration of a triple-lipid mixture of barrier lipids, which normalizes LB production and barrier function during PS/GC, also normalized epidermal AMP expression. Together, these results 1) provide a mechanistic basis for the adverse effects of PS/GC on cutaneous antimicrobial defense; 2) could explain the low, constitutive expression of these AMPs in normal epidermis; and 3) suggest that appropriate topical lipid formulations could normalize epidermal AMP levels during PS/GC.

Stress, Connective Tissue, and Wound Healing

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Control and effect of glucocorticoid metabolism within the skin

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It is a widely held belief that stress causes premature ageing and that this is as true for appearance and for skin ageing as for other health outcomes. To date however, the scientific evidence for such a belief is lacking and support for this view remains anecdotal. Since it is the fibroblast which is largely responsible for the production and maintenance of the extracellular matrix and collagen network within the dermis, we have investigated the synthesis and control of glucocorticoid levels e.g. cortisol, by this cell type.
Cortisol can either be produced from cortisone or synthesised de novo through a series of enzymatic transformations from cholesterol. By studying, the level & activity of the enzymes involved (and therefore the level of cortisol) we have gained insights into the local control of glucocorticoid levels within the skin. In turn we have examined the effect of this local control upon the functional activity of the dermal fibroblasts.

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Chronic stress and its role in skin ageing

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Neuroendocrine stress resulting from every day life occurrences has been shown, through longitudinal studies, to be a key driver of age-associated conditions. The impact of psychological stress on skin appearance and the underlying biological mechanisms however, is a relatively understudied area to date. Despite there being a great deal of anecdotal evidence on the detrimental effects of long-term stress on skin, little research has been reported with respect to changes in skin physiology and function. Therefore, we have examined the impact of stress on changes of key markers of skin ageing, in particular, measuring variations in inflammation and dermal matrix remodelling. In the first instance, we have used an *in vitro* model to investigate the effects of chronic Glucocorticoid treatment on Human umbilical vein endothelial cells and measured changes in InterCellular Adhesion Molecule 1 expression. Chronic Glucocorticoid treatment of Human umbilical vein endothelial cells resulted in an exacerbation of both InterCellular Adhesion Molecule 1 and Interleukin 6 expression. Furthermore, examination of the role of neuroendocrine stress on the dermal matrix, using chronically Glucocorticoid treated fibroblasts, resulted in an inhibition of Matrix Metalloproteinase-1, Procollagen I and IL-6 synthesis and secretion. The data presented in this paper provides new evidence to suggest that chronic stress may result in an exacerbation of inflammation and inhibition of dermal matrix remodelling. This stress-induced inhibition of dermal matrix repair, along with elevated inflammatory cell recruitment, will contribute significantly to the ageing of the skin.

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The role of fibroblast growth factor receptor 2 gene in the pathogenesis of venous leg ulcer

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Fibroblast growth factor receptor 2 gene (FGFR2) codes for tyrosine kinase receptors: keratinocyte growth factor receptor (KGFR = FGFR2-IIIb), mainly expressed by keratinocytes and bacterially expressed kinase (BEK = FGFR2-IIIc), expressed by mesenchimal and endothelial cells. Both isoforms play pivotal roles in wound healing. The aim of our study was to identify single nucleotide polymorphisms (SNPs) of the FGFR2 gene, which may play a role in the pathomechanism of prolonged wound healing in patients with leg ulcer and to investigate the expression pattern of FGFR2-IIIb in keratinocytes of patients with venous leg ulcer. SNP analysis was performed with mutation specific TaqMan probes and quantitative real-time RT-PCR was used for mRNA expression studies. Comparing the SNP data of leg ulcer patients ($n = 82$) with healthy individuals ($n = 82$) we found an SNP located in the 3′ untranslated region (UTR) of FGFR2 (2451AG; 900 bp downstream from the ORF), which showed a significant difference in the allelic distribution between leg ulcer patients and healthy individuals ($p = 0.0103$). We could also demonstrate that the mRNA expression level of FGFR2-IIIb in cultured keratinocytes isolated from the epidermis of leg ulcer patients ($n = 8$) was approximately 4 times lower compared to healthy individuals ($n = 10$). We hypothesize that the abnormal functioning of the FGFR2 gene in leg ulcer contributes to the pathogenesis of the disease: the 3′ UTR SNP might alter the stability of the mRNA, resulting in decreased amount of FGFR2 protein and receptor dysfunction.

Stress and Skin Disorders

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Sustained improvements in symptoms of depression and other patient reported outcomes for up to 48 weeks in chronic plaque psoriasis patients treated with etanercept

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This 144-week, phase 3 study included a 12-week, double-blind, randomized phase with etanercept (ETN) 50 mg twice weekly (BIW) v placebo (PBO) in chronic moderate to severe plaque psoriasis (PsO) patients followed by 132 weeks of open-label ETN therapy that included 96 weeks at 50 mg BIW. Patients with a history of psychiatric disease were excluded from this trial. From the randomized phase, 288 (94%) PBO and 304 (98%) ETN patients entered the open-label phase and received ETN 50 mg BIW; 264 (92%) PBO and 274 (90%) ETN patients completed 36 weeks for a total of 48 weeks on study. Symptoms of depression were assessed using the Hamilton Rating Scale for Depression (HAM-D; no depression = 0-6, mild = 7–15, moderate-severe = 16–53) and the Beck Depression Index (BDI; minimal depression = 0–9, mild = 10–16, moderate-severe = 17–63). Improvement of $\geq$50% over baseline defined a responder for both HAM-D and BDI. Dermatology Life Quality Index (DLQI), scored 0 to 30 with lower score = better disease-specific quality of life, was used to assess various aspects of disease-specific quality of life. DLQI and subscale scores for symptoms/feelings and personal relationships are reported here. All analyses used last observation carried forward to account for missing data with $\leq$10% attrition from each group. HAM-D scores at baseline were equivalent for both groups. By week 12, the ETN group had improved significantly and showed a greater proportion of responders (43% v 32%, $p = 0.0048$). The PBO group achieved similar responses to the ETN group after 12 weeks of ETN (study week 24) and sustained these responses through week 48. BDI followed a similar response pattern to HAM-D. After 12 weeks of ETN, results were similar and sustained through week 48. DLQI was equivalent for both groups (12.1 v 12.5, ETN v PBO) at baseline. These diverged over the first 12 weeks (9.2 v 3.5, PBO v ETN; $p < 0.0001$); the difference disappeared by week 24 (3.5 v 2.9, PBO v ETN). The personal relationships subscale showed improvement; however, with subsequent ETN therapy, the PBO never achieved the same response as the ETN group (51.5% v 40.4%, ETN v PBO at week 48). The symptoms and feelings subscale showed a significant difference between groups as early as week 1; the 2 groups looked similar by week 24 and sustained this response through week 48. With etanercept 50 mg twice weekly, chronic moderate to severe plaque psoriasis patients showed improvements in patient-reported outcomes, including symptoms of depression as measured by HAM-D and BDI , in both double-blind and open-label phases of this clinical trial and sustained these improvements for up to 48 weeks.

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Variable pulsed light (VPL) reduces treatment-induced pain in patients undergoing photodynamic therapy for actinic keratoses

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Background: Photodynamic therapy (PDT) is a well documented and established treatment alternative for epithelial skin cancer like basal cell carcinoma and actinic keratoses (AK). PDT induces selective tissue necrosis that occurs upon illumination with red light; this based upon the induction of reactive oxygen species upon activation of synthesized porphyrins. Unfortunately, free nerve endings are co-stimulated during this process thus inducing pain which sometimes leads to treatment interruption. The purpose of this study was to investigate a modification of the illumination process using a variable pulse light source (VPL™, Energist Ultra, Energist, U.K.) with spectral characteristics matching the absorption spectrum of the photosensitizer.
Method: A randomized parallel-group trial was conducted. A total of 25 patients (8 f, 17 m, mean age 73 yrs) were included suffering from actinic keratoses (AK) on the skin and the scalp. Methyl aminolevulinate (MAL, Metvix, Galderma, France) was applied on the targeted area for 3 hrs, subsequently one side received an illumination with a LED light source (37 J/cm^2, duration 12 min), and the contra lateral side received 80 J/cm^2 (double pulsed @ 40 J/cm2) with VPL™, with a pulse train of 15 impulses each with duration of 5 ms utilising a 610–950 nm filtered handpiece. Therapeutic outcome was evaluated using a lesion score after 2 weeks and 3 months; pain assessment was made after each treatment side with the use of a visual analogue scale (VAS).
Results: At beginning of the treatment patients showed an overall of 238 AK on face and scalp. After two weeks and three months, there was no significant difference between the therapeutic outcomes using the different illumination systems. However, pain assessment immediately after PDT revealed a significant lower pain level (4.3 vs. 6.4) for the VPL™ treated side.
Conclusions: The use of short pulsed light (variable pulsed light) is thus an efficient and useful alternative in the photodynamic treatment of AK where otherwise pain development can be a limiting factor for the performance of PDT.

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Stress response in a mouse model of alopecia areata

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This study aimed to investigate the effects of stress on the pathogenesis of alopecia areata (AA), a nonscarring hair loss disease, using the C3H/HeJ mouse model. Normal (n = 36) and AA affected (n = 36) mice were examined immediately after (basal) and 30 mins after (stressed) 20 sec exposure to light ether anesthesia (experiment 1), or prior to (basal) or immediately following 1 (acute stress) or 5 (repeated stress) exposures to 30 min restraint stress (experiment 2). Trunk blood was collected for stress hormone measurement and hippocampi were dissected for measurement of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) levels by quantitative RT-PCR. Results showed that under basal conditions, AA mice had corticosterone (CORT) levels similar to control animals. In contrast, AA mice had significantly blunted CORT and adrenocorticotropic hormone (ACTH) responses to acute ether stress, primarily a physiological stressor, but not to acute restraint stress, primarily a psychological stressor. After repeated restraint stress, CORT responses of normal mice were decreased due to habituation, but AA animals continued to have elevated CORT level. Moreover, following ether stress, AA animals had an almost 2-fold increase in hippocampal GR expression compared to normal animals. These results indicate that AA mice have significantly blunted responses of both CORT and ACTH to an acute physiological stressor and less adaptive responses to a repeated psychological stressor. This failure to respond to acute physiological stress suggests a possible increase in vulnerability to pro-inflammatory activity, which may play a role in the pathogenesis of AA. The failure to habituate to repeated psychological stress may also indicate an inadequate capacity for adaptation in AA affected rodents. The increased level of hippocampal GR expression may lead to a change in feedback regulation of HPA activity. Together, these results suggest that altered hypothalamic-pituitary-adrenal (HPA) may play a role in vulnerability to disease in AA mice.

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Stress and seborrheic dermatitis

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Stress is known as a triggering factor of seborrheic dermatitis but there is no published study confirming this fact. We have performed a prospective study for 4 months, by submitting a self-questionnaire to outpatients.

82 patients were included in the study: 46 men and 36 women. The middle age was 45.24 years (from 18 to 82). Scalp was involved in 82%, face in 33%, chest in 20% and other areas in 13%. There was no patient with Parkinson disease, HIV disease or cancer. 5% declared psychiatric disorder but 11% took a psychotrope during the study (neither lithium nor neuroleptic). Among usual triggering factors, 56% patients spontaneously reported stress. The factor triggering the first eruption was unknown in 72% and stress was noted by 28%. About the present eruption, the data were respectively 46% and 48%. 50% people reported a stress event during the week preceding the eruption and 84% in the month. No correlation was found between this report and the presence of anxious symptoms or an anxious personality. Psychological repercussions of the disease were assessed as nul by 26%, weak by 20%, moderate by 35% or severe by 11%. Concerning the ongoing eruption, the data were: nul 35%, weak 19%, moderate 19% and severe 6%.
In this population, with a low number of psychiatric disorders, stress was cited by more than 50% of cases as a usual triggering factor for the disease and as a triggering factor for the ongoing eruption. Psychological repercussions do not appear severe but are present. Our study seems to confirm the role of stress in the pathophysiogeny of seborrheic dermatitis. But there is no association of the role for stress and an inadequate psychological response to stress.

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Concept of a 3 month adjuvant participatory program for patients with chronic dermatoses

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In our experience, there is a general trend in maximalizing pharmacological therapy in chronic dermatoses. On the other hand, these pharmacologic treatments are not always fully efficient. Or for different reasons, some patients are not receiving full satisfaction in terms of their expectations for results of treatment. Therefore, a project that would offer our patients the opportunity to participate more fully in their own health care will be set up in the Dermatology of the Ghent University Hospital. This could not only result in a better quality of life, but also be pharmaco-economically positive.

Here I want to present for discussion the set up of a total care program. Patients with chronic skin diseases where a psycho-immuno-neurologic etiology might play such as psoriasis, atopic dermatitis, hyperhidrosis, telogenic effluvium, idiopathic itch, lichen simplex, rosacea, alopecia areata, vitiligo, seborrheic dermatitis, acne, and chronic urticaria will be screened for inclusion. They will follow a 3 month program – next to a classical medical treatment - in which the following items will be included: education session from a professional about the skin disease itself (etiology, prognosis…) – psychotherapy group sessions teaching behavioral stress reduction techniques – mindfulness meditation – physical training and yoga – dietary, sleep and anti-addiction advise and a specific (cosmetic) skin care education workshop 'hands on'. During the program 2 evaluation consultations with the dermatologist will be included and questionnaires concerning qualtiy of life will be obtained. 3 months after stop of the program patients will be invited again to see whether the patient is able to cope better with his/her skin disease, whether the disease free intervals increase, whether he/she continues the life style techniques in a more independent way, after this period.

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The Japanese version of Cutaneous Body Image scale: Translation and its cross-cultural adaptation

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Cutaneous body image is defined as the individuals' mental perception of the appearance of their skin, hair, and nails. The concept of cutaneous body image is an important psychodermatological element both in skin diseases that can affect the patients' appearance and in body dysmorphic disorders. To measure individuals' cutaneous body image, practical and accurate instrument is necessary. In this study, we translated into Japanese, Cataneous Body Image scale, 7-item instrument originally created by Gupta *et al*. (*J Invest Dermatol*. 2004;123:405–6) using forward- and back-translation method. Five bilingual persons whose primary language was Japanese translated the instrument into Japanese and produced a unique translation by consensus. Two bilingual persons whose primary language was English carried out back-translations of the first Japanese version. The original Canadian author of the instrument reviewed both of the first back-translations. Four doubtful items required a second back-translation to reach satisfactory agreement with the original instrument. The Japanese version was pre-tested in a pilot group composed of Japanese adults with and without skin diseases and revealed to be comprehensive and have no problematic item for adaptation to Japanese culture. In conclusion, we have developed a semantically equivalent translation of Cutaneous Body Image scale into Japanese. The instrument seemed to be practical and useful to measure cutaneous body image of Japanese dermatological patients, though further evaluation of the measurement property is necessary.

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Psychophysiological stress mechanisms in psoriasis: a prospective study

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Objective: There is increasing evidence indicating that psychological stress factors, such as interpersonal stressors and perceived stress, contribute to chronic inflammatory diseases, such as psoriasis. However, the specific stress-disease-relationships as well as psychophysiological mediating factors, including immune and neuroendocrine functions, have only very incidentally been investigated.

Method: To examine psychophysiological stress mechanisms, a prospective study is performed in 100 patients with psoriasis, with weekly and monthly assessments of clinical, laboratory and self-report data during a period of 6 months. In addition to shorter- and longer-term self-report measures of psychological stressors, data on clinically assessed disease activity as well as immune and neuroendocrine measures of blood samples are collected.

Results and conclusions: Preliminary results of the prospective relationships between stress measures and measures of disease activity and physical symptoms in 27 patients with psoriasis supported the expected stress-disease relationships. Particularly, results showed that measures of perceived and interpersonal stress were predictive for disease-related changes of disease activity in physical symptoms of itch during the next weeks. To study possible common effects in different chronic inflammatory diseases, the same relationships were examined in patients with rheumatoid arthritis, using the same research design. Findings were overall in line with the results in psoriasis and supported the idea that common stress factors contribute to inflammatory activity in different chronic inflammatory diseases, including psoriasis.

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Central sensitization in chronic itch and pain: Generalized and symptom-specific mechanisms

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Objective: Central sensitization of physical symptoms (i.e. central-controlled sensory sensitivity) is assumed to be primarily responsible for high symptom reports of patients, independently of a known pathophysiological etiology. Particularly in patients suffering from chronic physical symptoms, such as chronic itch, central sensitization is assumed to be involved in the maintenance and increase of physical symptoms in the long term. In line with basic psychophysiological theories, two central sensitization processes are assumed to be disregulated in patients with chronic physical symptoms, resulting in 1. generalized sensitization: the tendency to report an overall lowered threshold to somato-sensory stimuli and 2. symptom-specific sensitization: the tendency to interpret an ambiguous sensory stimulus in correspondence to the patient's main physical complaint (e.g. itch in chronic itch).

Method: To get insight into generalized and symptom-specific sensitization phenomena, different sensory stimuli of quantitative sensory testing QST (tactile and electrical stimulation) were applied in patients with atopic dermatitis suffering from chronic itching, patients with fibromyalgia suffering from chronic pain, and healthy controls. Intensities on pain and itch were assessed separately on a numeric rating scale.

Results: Both patients with chronic itch and patients with chronic pain had significantly lower tolerance thresholds for the different noxious stimuli in comparison to healthy controls. However, patients with chronic itch reported more itch, while patients with chronic pain reported more pain in comparison to the other patient group and the healthy controls.

Conclusions: Results suggest that both, generalized and symptom-specific sensitization processes, play a role in patients with chronic physical symptoms of itch and pain.

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Dermatological manifestations (DeMs) associated with chronic fatigue syndrome (cfs): two case reports

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DeMs have been described in 10–35% of patients with CFS, but a better description of such lesions is awaited. Two ladies with complaints fulfilling the major criteria and at least 6 minor criteria for the diagnosis of CFS consulted the author because of concurrent DeMs. Patient A (PtA, aged 40) presented with recalcitrant idiopathic palmoplantar pompholyx and anogenital pruritus; patient B (PtB, aged 45) with chronic telogen effluvium. In both cases, DeMs and CFS evolved and worsened synchronously, and the key triggering factors were overtly hard work and family-related stress. Curiously enough, the diagnosis of CFS was intermittently warranted, as both patients, who were engaged in successful, self-fulfilling, but highly demanding careers, wavered between search of help and harsh denial of subjective fatigue symptoms (''I can't afford to have CFS''). CFS-related complaints had started some six months (PtA) or two years (PtB) before the first consultation with a flu-like illness, which had serologically proved to be infectious mononucleosis in PtA. Repeated clinical, lab & imaging evaluations ruled out organic diseases, rather they disclosed somewhat typical patterns of ''aspecific'' immune dysfunctions such as slight ANA-positivity, higher-than-normal serum IgG, or fluctuating absolute lymphocytosis. The psychological profiles and psychiatric histories were highly significant (PtA: grown up in a problematic family, with ambivalent dominant-abandoning parental figures; features of a moderate mixed personality disorder; PtB: slight obsessive-compulsive disorder; current marital troubles in both cases). Cognitive-behavioral and informal psychotherapy coupled with intermittent low-dose hydroxyzine (PtA) or fluvoxamine (PtB) appeared superior to either standard or off-label dermatological treatments. Several dermatological diseases are known to be worsened by ''stress''. From a clinical and therapeutic viewpoint, it is of paramount importance that unspecific (or ''existential'') stress be distinguished from symptomatic stress inscribed in definite nosological subsets. Such patients need continuous expertise and follow-up, and should not be ''left alone'' with their intricate psychocutaneous conditions.

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Lower serum concentration of dehydroepiandrosterone sulphate in patients with chronic idiopathic urticaria (CIU)

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Evidence proves the immunomodulatory function of androgens. It is known that relative deficiency of dehydroepiandrosterone sulphate (DHEA-S) is associated with various acute inflammatory stressful disorders and chronic inflammatory diseases. In addition, stress is thought to play an important role in CIU pathogenesis and modulates secretion of DHEA-S. Therefore, the present study investigated whether there occur any changes in circulating concentration of this androgen in patients suffering from CIU. Serum concentration of DHEA-S was measured by the electrochemiluminescence immunoassay (ECLIA) method in 31 male and 40 female patients with CIU and in 60 sex-, age-, weight- matched healthy controls. In CIU patients, serum concentration of DHEA-S was statistically significantly lower as compared to the control subjects (males: 259.7 ± 131 *versus* 373.5 ± 126 (mean $\pm$ SD) μg/dl, respectively; females: 160.8 ± 115 *versus* 245.6 ± 124 μg/dl, respectively; $p < 0{,}05$ U Mann-Whitney test). No significant association was found between DHEA-S concentration, CIU duration and the intensity of the disease. It seems that declining circulating concentration of DHEA-S is a phenomenon accompanying CIU, but it is unknown whether this alteration plays a part in the etiopathogenesis of the disease, or is a consequence of it and other factors, including stress.

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Effect of cortcotropin-releasing factor on psychological stress-induced scratching behavior in atopic dermatitis model mice

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Atopic dermatitis is a common pruritic inflammatory skin disease. It has been already established that environmental factors such as mites or dusts exacerbate the dermatitis. Furthermore, psychological stress has also been regarded as an exacerbating factor. However, there are very few reports verifying the relationship between psychological stress and diseases including AD experimentally or scientifically. We have demonstrated that psychological stress by itself can trigger atopic dermatitis-like skin lesions in atopic dermatitis model mice, NC/Nga exposed to water avoidance stress (WAS) under specific-pathogen free (SPF) condition. In this study, we aimed to investigate the effect and mechanism of corticotropin-releasing factor (CRF) on psychological stress-induced scratching behaviour.

[Materials & methods] Under SPF condition, NC/Nga mice were exposed to WAS (for 60 minutes daily 5 days a week) for 4 weeks. After exposed to stress, mice were maintained in their cages. Furthermore, to determine the effect of CRF in this stress response, the mice received CRF (50 μg/kg) intraperitoneally 30 minutes before WAS in the same protochol. We also examined the effect of CRF receptor antagonist in this stress response. We monitored scratching behaviour during psychological stress, and measured the levels of serum IgE, serum corticosterone, fecal pellet output.

[Results] Under SPF condition, psychological stress elicited atopic dermatitis-like skin lesions along with the increased level of serum IgE, corticosterone, and fecal pellet output in NC/Nga mice. CRF suppressed scratching behaviour induced by WAS. On the other hand, neither CRF receptor antagonist nor substance P antagonist did suppress scratching behaviour by WAS. Our data indicate that CRF may suppress atopic dermatitis-like skin lesions that were triggered by psychological stress via CRF receptor.